



NRL/MR/6110--06-9005

Impact of a Hydrate-Based Marine Desalination Technology on Marine Microbiota and Water Quality

RICHARD B. COFFIN

MICHAEL T. MONTGOMERY

CHRISTOPHER L. OSBURN

*Chemical Dynamics and Diagnostics Branch
Chemistry Division*

December 27, 2006

Approved for public release; distribution is unlimited.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 27-12-2006		2. REPORT TYPE Memorandum Report		3. DATES COVERED (From - To) Lab report: Marine Desalination	
4. TITLE AND SUBTITLE Impact of a Hydrate-Based Marine Desalination Technology on Marine Microbiota and Water Quality				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Richard B. Coffin, Michael T. Montgomery, and Christopher L. Osburn				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Laboratory 4555 Overlook Avenue, SW Washington, DC 20375-5320				8. PERFORMING ORGANIZATION REPORT NUMBER NRL/MR/6110--06-9005	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Global (ONRG) One Liberty Center (Suite 1426) 875 North Randolph Street Arlington, VA 22203-1995				10. SPONSOR / MONITOR'S ACRONYM(S)	
				11. SPONSOR / MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water. To scale this technology to support different country needs there is a requirement to assess the environmental impact of desalination. The NRL MDS collaboration on biological influence of marine desalination focuses on water quality of processed water and environmental impact of the marine desalination application. With this segment of the project accomplished, the subsequent evaluation will focus on the impact of large scale marine desalination on environmental quality. To assess the environmental impact of the marine desalination system there is a need to understand resulting changes to key bacterial and elemental cycles. Seawater vertical transport, alteration of water flow, and filtration have potential to change the microbial community diversity, substrate availability, electron acceptors and key element speciation. Microorganisms surveyed in this analysis control elemental cycles that set environmental quality for the entire aquatic food chain. This study initiates assessment of the effect of the MDS process on water quality and the growth rate of the natural bacterial assemblage. We measured heterotrophic bacterial production, dissolved organic carbon (DOC) concentration and stable isotope values, and fluorescence spectra of the organic matter that partition into the hydrate and waste water streams.					
15. SUBJECT TERMS Hydrate Methane Water desalination Carbon dioxide					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UL	18. NUMBER OF PAGES 22	19a. NAME OF RESPONSIBLE PERSON Richard B. Coffin
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) (202) 767-0065

IMPACT OF A HYDRATE-BASED MARINE DESALINATION TECHNOLOGY ON MARINE MICROBIOTA AND WATER QUALITY

Lab Report: Marine Desalination
Sponsor: ONR, Paul Armistead

Richard B. Coffin
Michael T. Montgomery
Christopher L. Osburn

Marine Biogeochemistry Section
Code 6114
Naval Research Laboratory

POC:
Richard B. Coffin
202-767-0065
rick.coffin@nrl.navy.mil

TABLE OF CONTENTS

Title Page	1
Table of Contents	2
Disclaimer	3
Introduction	4
Methods and Materials	5
Sampling	5
Synchronous fluorescence	5
Dissolved organic carbon and stable isotope composition	5
Tangential flow filtration	5
Dissolved inorganic carbon	5
Heterotrophic bacterial production	5
Marine Microbiota	6
Results and Discussion	6
Water Quality	6
Marine Microbiota	7
References	8
List of Figures	10

ii. DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability of responsibility for the accuracy, completeness, or usefulness of any information, apparatus product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

INTRODUCTION

Shortages of potable water affect 88 developing countries where 80-90% of all diseases and 30% of all deaths result from poor water quality (Leitner 1998, see also review by Miller 2003). Water short fall in many countries is at the level of petroleum availability. With the current predictions of global population expansion this problem will increase the need for a solution. One strategy for generation of high quality potable water involves desalination via clathrate crystal formation. Forty years ago, the US Department of the Interior supported construction of two freeze desalination pilot plants that were based on clathrate formation (CCl_2F_2 , and butane) (Miller 2003). Dendritic hydrate crystal formation made it difficult to separate the freshwater crystals from the aqueous brine (Barduhn 1982). A Bureau of Reclamation study (McCormack and Andersen 1995) was followed by a partially successful pilot test conducted at the Natural Energy Laboratory of Hawaii (McCormack and Niblock 1998, Miller 2003). The primary issues still involved clathrate crystal separation which led to a subsequent program to determine the filterability of crystals, the surveying alternate higher temperature clathrate formers and design and operation of a wash column (McCormack and Niblock 2000, Miller 2003).

Marine Desalination Systems (MDS) is currently developing new technology in gas hydrate formation to supply potable water. To scale this technology to support different country needs there is a requirement to assess the environmental impact of desalination. It is important to determine the MDS technology impact on local water quality and marine microbiota through partitioning of dissolved organic carbon and brine production in wastestreams. High local fluctuations in salinity that would inevitably result from the hydrate formation may be comparable to those seen in sea ice formation. The internal environment of sea ice is known as the brine channel system and is characterized by low temperature, high salinity and elevated concentrations of dissolved nutrients and dissolved organic carbon (Kaartokallio et al. 2005). These changes are known to affect the composition of estuarine and marine bacterial assemblages (Ezura et al. 1974, Rheinheimer 1977) and particularly psychrophilic marine bacteria (Helmke and Weyland 1995, Morita 1976, Morita et al. 1973, Stanley and Morita 1968). The latter class of bacteria may be important in the expected deep water hydrate formation zone for the MDS technology. Bacteria change the composition of their cell membranes in response to local environmental conditions and these alterations can make them more or less susceptible to drastic temperature and salinity gradients (Nichols et al. 2000). These unique environments lead to differences in bacterial community structure between sea ice assemblages, such as those in the hydrate, and those in the open water, such as those that might be produced in the process streams of the MDS technology (Del Giorgio and Bouvier 2002, Kaartokallio et al. 2005). The bacterial community in naturally occurring hydrate formations has been reported to harbor unique eubacteria and archebacteria genotypes not reported elsewhere (Reed et al. 2002, Mills et al. 2005) including methanotrophic *Archaea* (Lanoil et al. 2001). Deep sea methane hydrate assemblages are known to be adapted to the elevated salinity that would be commensurate with hydrate formation coupled with the generation of interstitial brine (Kaye and Baross 2004). These microorganisms are responsible for key elemental cycles in nature that affect the ecosystem health.

This study initiates assessment of the effect of the MDS process on water quality and the growth rate of the natural bacterial assemblage. We measured heterotrophic bacterial production, dissolved organic carbon (DOC) concentration and stable isotope values, and fluorescence spectra of the organic matter that partitions into the hydrate and waste water streams.

MATERIAL AND METHODS

Sampling

Samples were collected in 250 mL Nalgene bottles previously acid-washed (2% HCl) and rinsed with MilliQ grade water. Samples for stable isotope analyses were collected in cleaned (acid-washed and then baked at 450 °C for 6 h) glass bottles using clean techniques (e.g., gloves). Atlantic Ocean seawater was collected from Indian River Inlet, DE on an incoming tide. MDS system source water was collected from Tampa Bay surface water. After operation of the MDS hydrate formation and dissociation system (Figure 1), water samples were collected from above the hydrate diaphragm (upstream) and below the hydrate (downstream). In addition, frozen hydrate crystals were collected and melted at room temperature (hydrate water).

Synchronous fluorescence

Seawater samples (3 mL) were used for Synchronous Fluorescence (SF) measurements on a Shimadzu RF5301 spectrofluorometer operating in the synchronous mode. Excitation wavelength range will be 236 to 600 nm with a 14 nm offset. All spectra acquired were calibrated to the water Raman signal and normalized to instrument corrections.

Dissolved organic carbon and stable isotope composition

Seawater samples (40 mL) were filtered through 0.2 μm (nominal pore diameter) Teflon filters into cleaned glass vials and sealed with Teflon-lined silicone closures. H_3PO_4 (85%) was added to adjust the pH of samples to ~ 3 and then samples were sparged for 10 min with UHP Helium. DOC measurement were conducted by heated (98°C) persulfate oxidation on an OI Analytical 1010 TOC analyzer employing an NDIR detector that measures CO_2 evolved from the oxidation. Potassium hydrogen phthalate was used to construct a calibration curve. Stable carbon isotopes of DOC ($\delta^{13}\text{C}$ -DOC) were measured on the evolved CO_2 that vents from the OI instrument. Teflon tubing connected to the 1010 vent carries the CO_2 to a 750°C reactor containing elemental copper and silvered cobaltous reagent to remove halogens. The scrubbed CO_2 was then re-focused using a Porapak GC column and transferred through a Conflo III interface to the inlet of a ThermoElectron DeltaPlusXP isotope ratio mass spectrometer (IRMS). $\delta^{13}\text{C}$ -DOC values was normalized to the VPDB international stable isotope standard of L-glutamic acid ($\delta^{13}\text{C}$ -DOC = -26.2‰).

Tangential flow filtration

A PALL MiniTan microcassette tangential flow filtration system was used to separate DOC compounds greater than 1000 Daltons. A peristaltic pump was used to recirculate 100 mL of water in the filtration system. SF, DOC, and $\delta^{13}\text{C}$ -DOC was measured on the filtrate and the retentates of the TFF system to estimate the changes to these properties induced by selective removal of higher molecular weight organic C compounds.

Dissolved inorganic carbon

Dissolved inorganic carbon was measured using a Somma coulometer (Johnson et al. 1987).

Heterotrophic bacterial production

The leucine incorporation method (Kirchman et al. 1985, Kirchman 1993, Smith and Azam 1992) was used to measure bacterial production as adapted by Montgomery et al. (1999). A 0.50 μL aliquot of wet surface sediment from each station was added to 2 mL centrifuge tubes (three experimental and one control) which were pre-charged with [^3H -4,5]-L-leucine ($154 \text{ mCi mmol}^{-1}$). The sediment was extracted from the benthic grab sample and added to the 2 mL tube using a 1 mL plastic syringe with the end cut off. One mL of 0.45 μm nom. pore dia. (Acrodisk, Gelman) filtered bottom water (collected <1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1-2 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam (1992). A constant isotope dilution factor of 1000 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). One mL syringed samples of wet sediment were dried at 50 $^{\circ}\text{C}$ and used to convert production values to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

RESULTS AND DISCUSSION

Water Quality

Salinity

The salinity of the seawater from the source (Tampa Bay) and various process streams (Figure 1) were determined for 12 experiments generating hydrates. The primary goal of the desalination process is to create low salinity hydrate by excluding sea salt ions from the hydrate matrix and partitioning them into the downstream waste water. Depending on the volume of the hydrate produced relative to that of the process water, downstream salinity may measurably increase. Over the course of the 12 experiments, salinity of the melted hydrate water ranged from 0 to 7 ‰ with 10 of the samples ranging from 0 to 3 ‰ (Figure 2). As expected, source water and upstream water ranged from 27 to 34 ppt and covaried. Oddly, salinity in the downstream water actually decreased slightly ranging from 27 to 31 ‰. It was expected that the downstream water would increase in salinity with formation of the low salinity hydrate.

pH

Source water pH ranged from 6.6 to 7.5 among the 12 experiments and was lower in upstream (5.5 to 6.5) and downstream (5.7 to 6.2) samples (Figure 3). In the melted hydrate water, the total pH range was much greater (4.7 to 6.4) with seven of the experiments ranging from 4.7 to 5.3 and four experiments ranging from 6.0 to 6.4 (Figure 3).

Dissolved organic carbon

DOC concentration increases from 2 to 4 mg C L^{-1} in the source water to 2 to 14 mg C L^{-1} in the hydrate (though all but one sample are 2 to 7 mg C L^{-1}) to 3 to 8 mg C L^{-1} in the upstream water and 5 to 12 mg C L^{-1} in the downstream water (Figure 4). Relative to the source water, DOC concentration increases in all media suggesting that the gas involved in hydrate formation (propane) or other compounds associated with the gas that is bubbled into the upstream water is contributing to DOC in the process streams. The $\delta^{13}\text{C}$ -DOC values in the source water range from about -24 to -26 (Figure 5). With the exception of the two anomalous hydrate values of -20, the range of $\delta^{13}\text{C}$ -DOC values for the upstream, downstream and hydrate samples are all lighter (-24 to -30) than that in the source water. When $\delta^{13}\text{C}$ -DOC values are compared with increasing DOC concentration, isotope values become lighter,

changing from -24 at 2 mg L⁻¹ to around -30 at 10 to 12 mg L⁻¹ DOC (Figure 6). Petroleum hydrocarbons, such as the gas that was used to form the hydrates, have $\delta^{13}\text{C}$ values around -28 to -30. These data suggest that the dissolved hydrocarbon gas used to form the hydrate was influencing both the DOC concentration and the stable carbon isotope values in the process water and hydrate. Before future experiments are conducted the source of the DOC will be identified and the system will be redesigned to prohibit this contamination. Hydrate formation has recently focused on carbon dioxide as the catalyst gas and this may avoid future experimental problem.

Synchronous fluorescence (SF) profiles of organic matter in coastal waters can indicate the presence of certain moieties common to either terrestrially derived carbon (allochthonous: fulvic and humic acids) or marine derived (autochthonous: phytoplankton and other primary producers) (Figure 7). SF emission spectra derived from the source water were typical for estuarine systems with both terrestrial and phytoplankton related signals (Figure 8). The upstream process water spectra maintain many of the features of the source water spectra, as expected, but add a fluorescence peak at around 270 nm. This feature is also seen with the downstream water spectra and is the only peak in the hydrate water spectra (Figure 8). This peak is most likely produced from the addition of the dissolved hydrocarbon gas used to form the hydrate. The hydrate also appears to have excluded much of the organic matter signature associated with the source water.

Marine Microbiota

Process water production

The MDS process can affect the natural bacterial assemblage in primarily two ways: metabolic rate (production) and assemblage composition. Changing water quality can impact the microbial assemblage by reducing microbial production which would result from lower organic substrate or through environmental stresses caused by changes in chemical speciation and composition. There was a large range in bacterial production, 26 to 157 $\mu\text{g C L}^{-1} \text{d}^{-1}$, in the source water samples among the five experiments in which this was measured (experiments #4, 8, 10, 11, 12), though this range is typical for estuarine waters (Figure 9). Samples from the process water were much lower in bacterial production ranging from 0.08 to 0.28 $\mu\text{g C L}^{-1} \text{d}^{-1}$ in the upstream water, 0.08 to 0.21 $\mu\text{g C L}^{-1} \text{d}^{-1}$ in the downstream water, and 0.10 to 0.35 $\mu\text{g C L}^{-1} \text{d}^{-1}$ in the melted hydrate water (Figure 9). The MDS process or the sample collection appeared to dramatically affect bacterial growth based on the difference in production between the source water and process water samples.

Toxicity

Subsequent experiments were designed to differentiate between changes in bacterial production related to sample shipment and storage prior to the measurement and those due to changes in some water quality parameter that generally affects bacterial growth. Bacterial production was measured on unamended Atlantic Ocean surface seawater (100% treatment) and compared with samples that had additions of process water (10, 50% treatments) and to the process water alone. If there were no water quality parameters in the process water that were inhibitory to marine bacteria, then there would be a linear relationship among the production values between the Atlantic Ocean water alone (100% treatment) and the process water (0% treatment) (dotted line; Figure 10).

There was little effect of the Tampa Bay source water on Atlantic Ocean water, that is, the production value was the sum of relative contributions of the source waters (Figure 10). Bacterial production of the Atlantic Ocean water was inhibited by addition of all three process water samples (downstream, upstream and hydrate waters) (Figure 10). This suggests that there was some feature or

component of the process water that was inhibitory to bacterial metabolism of natural marine assemblages. Among the possible inhibiting factors is pH, low dissolved oxygen, and dissolved hydrocarbons though the 10% decrease in dissolved oxygen that would result from the 10% process water addition would not be likely to so dramatically decrease bacterial production in the Atlantic Ocean water. Likewise, given the buffering capacity of seawater, the pH change might not be enough to inhibit production to the extent seen in this experiment (Figure 10).

Future experiments will combine measurements of microbial community diversity, production and growth efficiency to assess the impact on the population and key elemental cycles.

REFERENCES

- Barduhn, A.J. 1982. Desalination by Freezing Processes, *In*, Encyclopedia of Chemical Processing and Design, McKetta and Cunningham, eds., Marcel Dekker, New York.
- Burdige, D. J., and C. S. Martens. 1990. Biogeochemical cycling in an organic-rich marine basin – 11. The sedimentary cycling of dissolved free amino acids. *Geochim. Cosmochim. Acta.* 54: 3033-3052.
- Del Giorgio, P.A., and T.C. Bouvier. 2002. Linking the physiology and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. *Limnol. Oceanogr.* 47:471–486.
- Ezura, Y., K. Daiku, K. Tajima, T. Kimura, and M. Sakai. 1974. Seasonal differences in bacterial counts and heterotrophic bacterial flora in Akkeshi Bay, p. 112–123. In R.R. Colwell and R.Y. Morita (ed.), *Effect of the ocean environment on microbial activities*. University Park Press, Baltimore, Md.
- Helmke, E., and H. Weyland. 1995. Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. *Mar. Ecol. Prog. Ser.* 117:269–287.
- Kaartokallio, H., M. Laamanen, and K. Sivonen. 2005. Responses of Baltic Sea ice and open-water natural bacterial communities to salinity change. *Appl. Environ. Microbiol.* 71(8):4364–4371.
- Kaye, J.Z., and J.A. Baross. 2004. Synchronous effects of temperature, hydrostatic pressure, and salinity on growth, phospholipid profiles, and protein patterns of four *Halomonas* species isolated from deep-sea hydrothermal-vent and sea surface environments. *Appl. Environ. Microbiol.* 70(10):6220–6229.
- Kirchman, D.L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In P.F. Kemp, B. F. Sherr, E.B. Sherr and J.J. Cole. *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Washington D.C. pp.509-512.
- Kirchman, D., E. K'nees, and R. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl. Environ. Microbiol.* 49: 599-607.
- Lanoil, B.D., Sassen, R., LaDuc, M.T., Sweet, S.T., and K.H. Nealson. 2001. Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates. *Appl. Environ. Microbiol.* 67(11):5143–5153.
- Leitner, G.F. 1998. Is there a water crisis? *Internat. Desalin. Water Reuse Quart.* 7 (1998) 10.
- McCormack R.A., and R.K. Andersen. 1995. Clathrate desalination plant preliminary research study, U.S. Bureau of Reclamation Water Treatment Technology Program Report No. 5.

- McCormack, R.A., and G.A. Niblock. 1998. Build and operate a clathrate desalination pilot plant, U.S. Bureau of Reclamation Water Treatment Technology Program Report No. 31, May.
- McCormack, R.A., and G.A. Niblock. 2000. Investigation of high freezing temperature, zero ozone, and zero global warming potential clathrate formers for desalination, U.S. Bureau of Reclamation Water Treatment Technology Program Report No. 59, June.
- Miller, J. E. 2003. Review of Water Resources and Desalination Technologies. National Nuclear Security Administration under Contract DE-AC04-94-AL85000, Sandia National Laboratories, Albuquerque, New Mexico, SAND REPORT, SAND 2003-0800
- Mills, H.J., Martinez, R.J., Story, S., and P.A. Sobecky. 2005. Characterization of microbial community structure in Gulf of Mexico gas hydrates: comparative analysis of DNA- and RNA-derived clone libraries. *Appl. Environ. Microbiol.* 71(6):3235–3247.
- Montgomery, M.T., T.J. Boyd, B.J. Spargo, R.B. Coffin, J.K. Steele, D.M. Ward, and D.C. Smith. 1999. Bacterial assemblage adaptation in PAH-impacted ecosystems. In B. C. Alleman and A. Leeson (eds.) *In Situ and On-Site Bioremediation*. Battelle Press, Columbus, OH, Vol. 5:223-228.
- Morita, R.Y. 1976. Survival of bacteria in cold and moderate hydrostatic pressure environments with special reference to psychrophilic and barophilic bacteria. *Soc. Gen. Microbiol. Symp. Ser.* 17:279–298.
- Morita, R.Y., L.P. Jones, R.P. Griffiths, and T.E. Staley. 1973. Salinity and temperature interactions and their relationship to the microbiology of the estuarine environment, p. 221–232. In L. H. Stevenson and R. R. Colwell (ed.), *Estuarine microbial ecology*. University of South Carolina Press, Columbia.
- Nichols, D.S., Olley, J., Garda, H., and R.R. Brenner. 2000. Effect of temperature and salinity stress on growth and lipid composition of *Shewanella gelidimarina*. *Appl. Environ. Microbiol.* 66(6):2422–2429.
- Reed, D.W., Fujita, Y., Delwiche, M.E., Blackwelder, D.B., Sheridan, P.P., Uchida, T., and F.S. Colwell. 2002. Microbial communities from methane hydrate-bearing deep marine sediments in a Forearc Basin. *Appl. Environ. Microbiol.* 68(8):3759–3770.
- Rheinheimer, G. 1977. Regional and seasonal distribution of saprophytic and coliform bacteria, p. 121–137. In G. Rheinheimer (ed.), *Microbial ecology of a brackish water environment*. Springer-Verlag, Berlin, Germany.
- Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* 51:201-213.
- Smith, D.C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^3H -leucine. *Mar. Microb. Food Webs* 6(2):107-114.
- Stanley, S.O., and R.Y. Morita. 1968. Salinity effect on the maximal temperature of some bacteria isolated from the marine environment. *J. Bacteriol.* 95:169–173.
- Tuominen, L. 1995. Comparison of leucine uptake methods and a thymidine incorporation method for measuring bacterial activity in sediment. *J. Microbiol. Methods*, 24:125-134.

LIST OF FIGURES

Figure 1. Schematic diagram of the MDS technology test system with source water (seawater) and location of samples from upstream, hydrate, and downstream water.

Figure 2. Salinity (‰) of source, upstream, downstream and three hydrate subsamples for 10 of the experiments.

Figure 3. pH of the Atlantic ocean seawater (ocean), Tampa Bay source water, and samples from upstream, downstream, and the melted hydrate water.

Figure 4. DOC concentration between the source, hydrate and upstream and downstream water during the hydrate formation for 8 of the experiments.

Figure 5. $\delta^{13}\text{C}$ -DOC values between the source, hydrate and upstream and downstream water during the hydrate formation for 8 of the experiments.

Figure 6. $\delta^{13}\text{C}$ -DOC values relative to total DOC concentration for all samples (source, upstream, downstream, and hydrate water).

Figure 7. Synchronous fluorescence in an estuarine water sample. The excitation wave lengths are presented for primary production, fulvic acids and humic acids.

Figure 8. Emission spectra (fluorescence intensity) for organic matter in source, upstream, downstream and hydrate waters.

Figure 9. Heterotrophic bacterial production (average (AVG) and standard deviation (SD) $\mu\text{C L}^{-1} \text{d}^{-1}$) compared in hydrate formation experiments between source, upstream, downstream and hydrate water.

Figure 10. Heterotrophic bacterial production ($\mu\text{C L}^{-1} \text{d}^{-1}$) mixed with different percentages of Tampa Bay source water, upstream and downstream water.

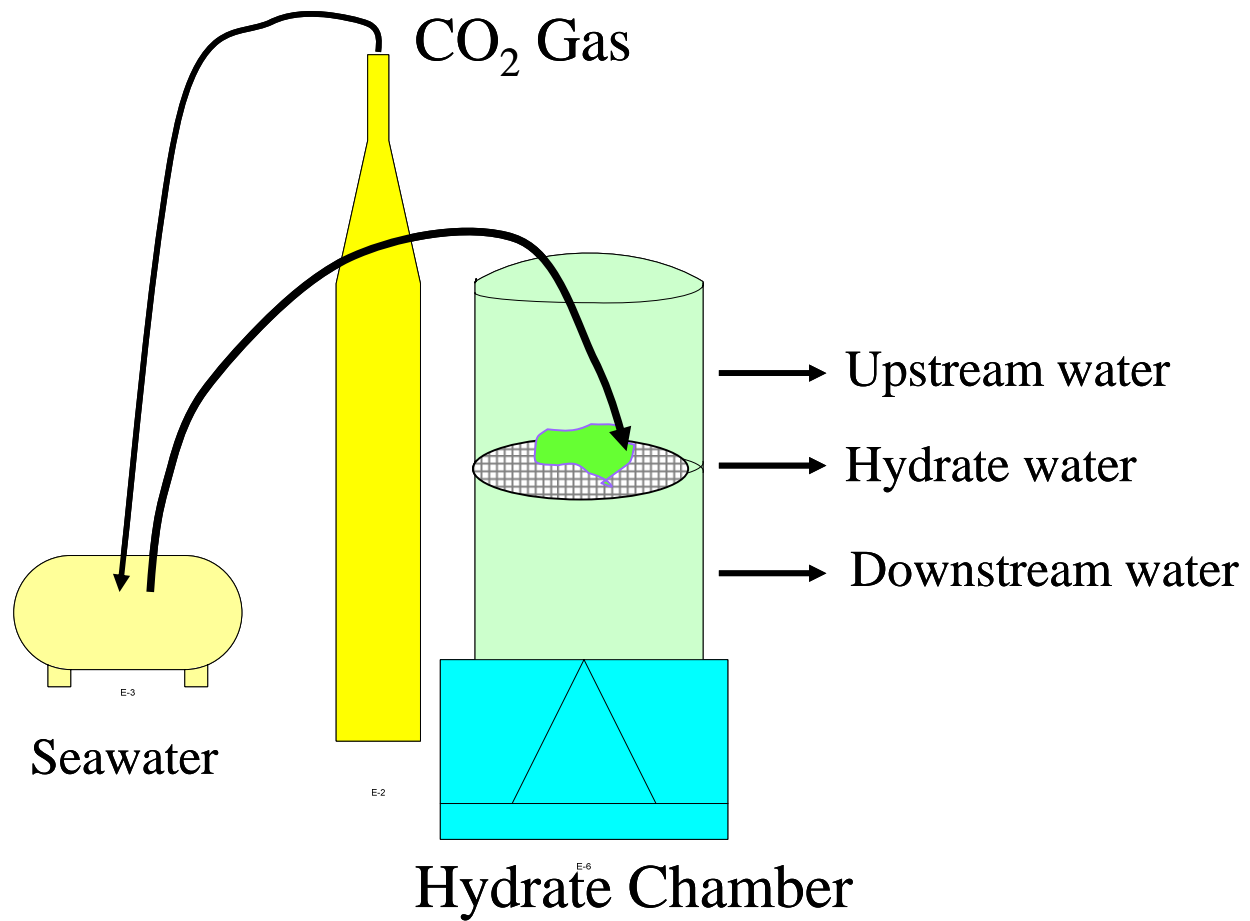


Figure 1. Schematic diagram of the MDS technology test system with source water (seawater) and location of samples from upstream, hydrate, and downstream water.

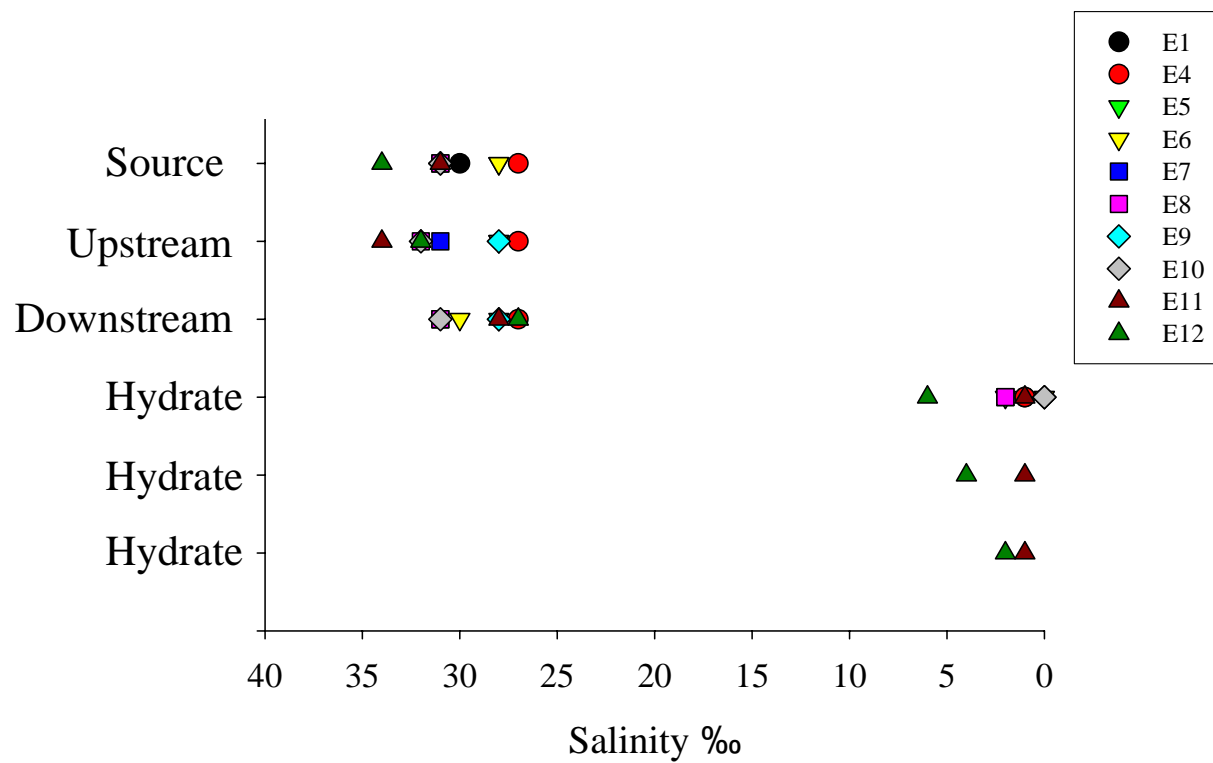


Figure 2. Salinity (‰) of source, upstream, downstream and three hydrate subsamples for 10 of the experiments.

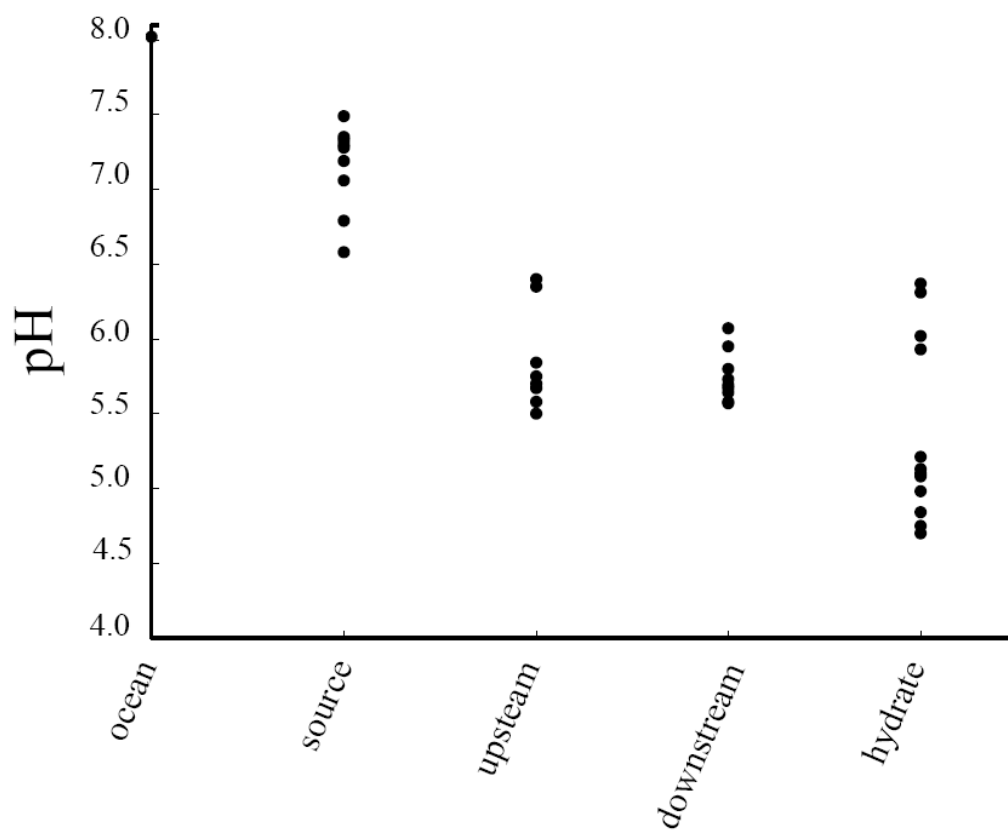


Figure 3. pH of the Atlantic ocean seawater (ocean), Tampa Bay source water, and samples from upstream, downstream, and the melted hydrate water.

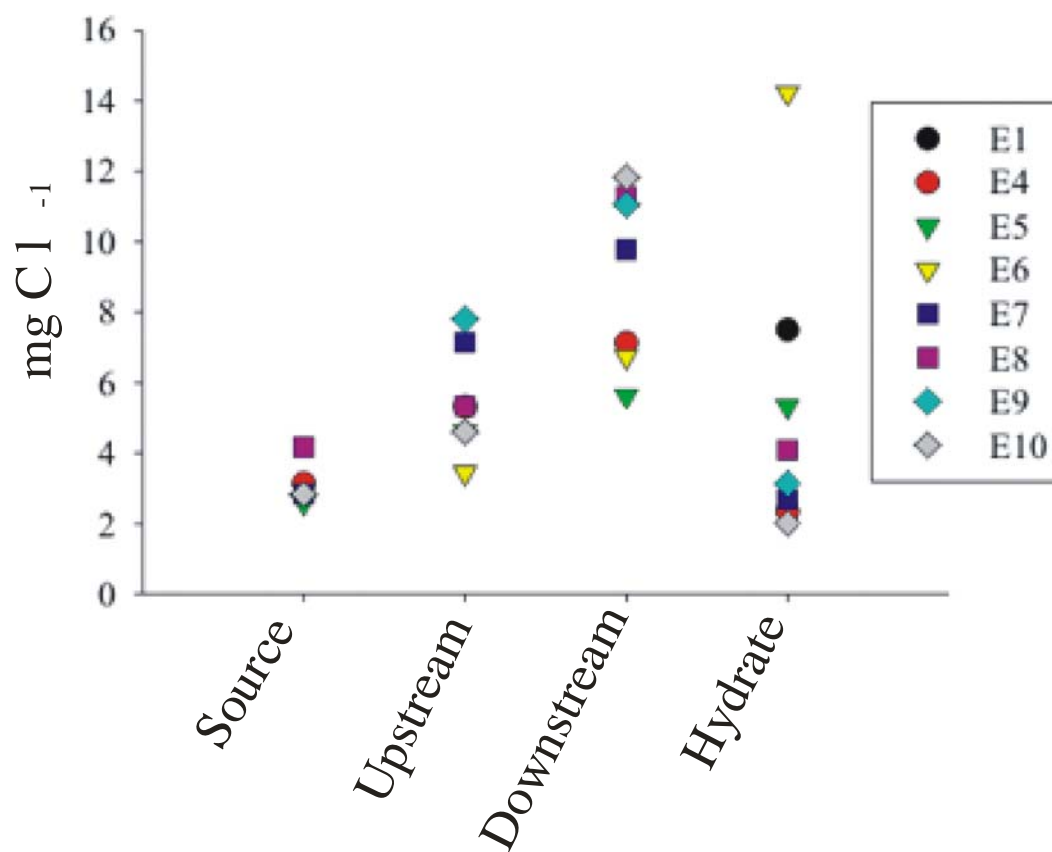


Figure 4. DOC concentration between the source, hydrate and upstream and downstream water during the hydrate formation for 8 of the experiments.

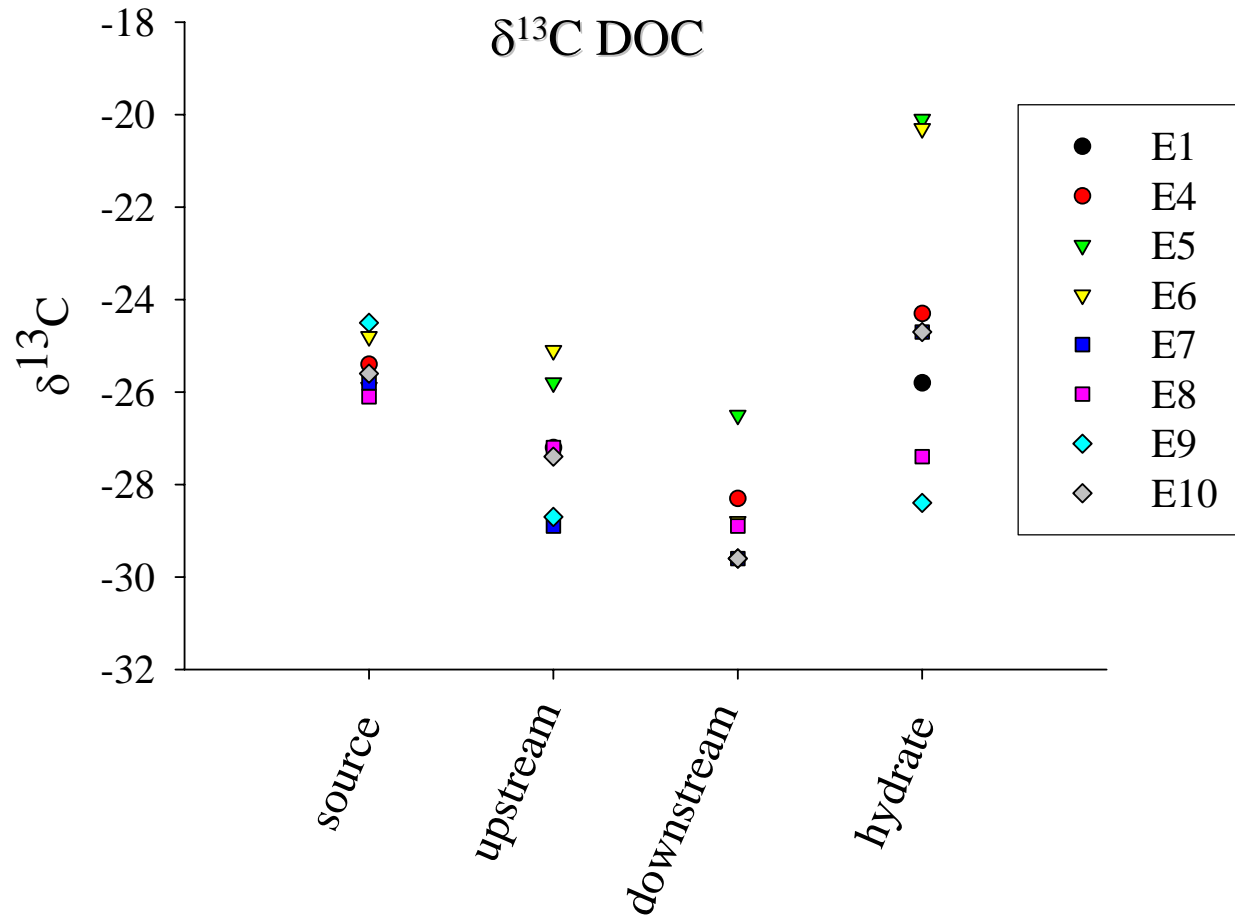


Figure 5. $\delta^{13}\text{C}$ -DOC values between the source, hydrate and upstream and downstream water during the hydrate formation for 8 of the experiments.

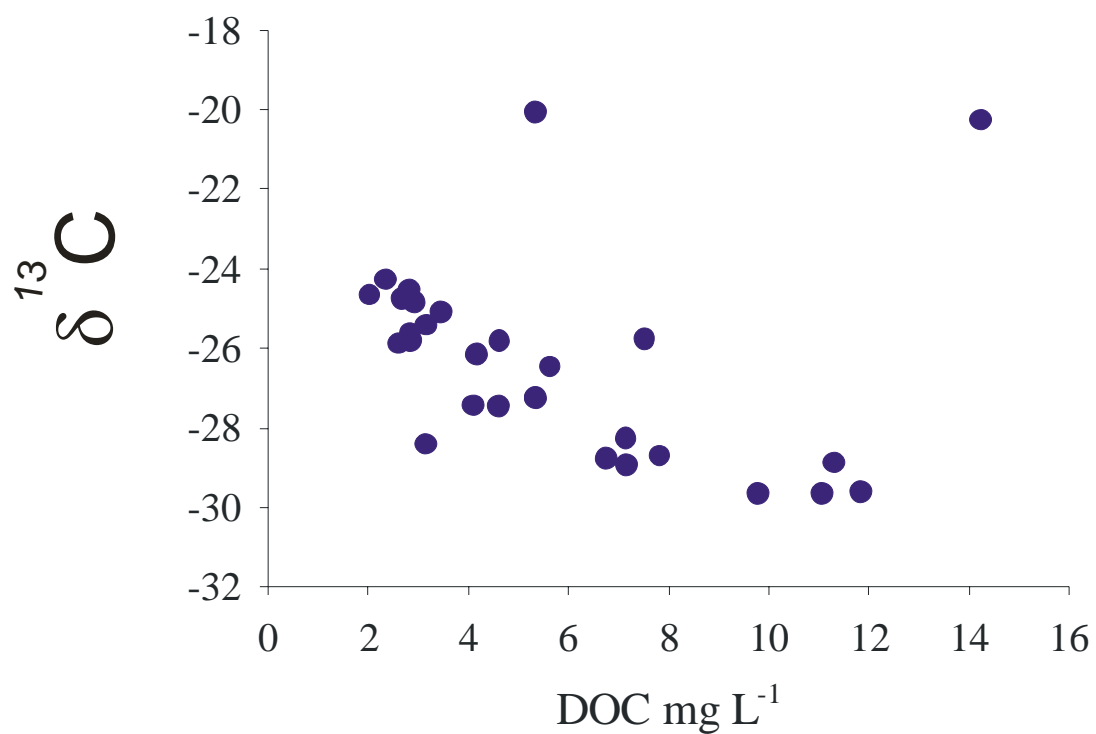


Figure 6. $\delta^{13}\text{C}$ -DOC values relative to total DOC concentration for all samples (source, upstream, downstream, and hydrate water).

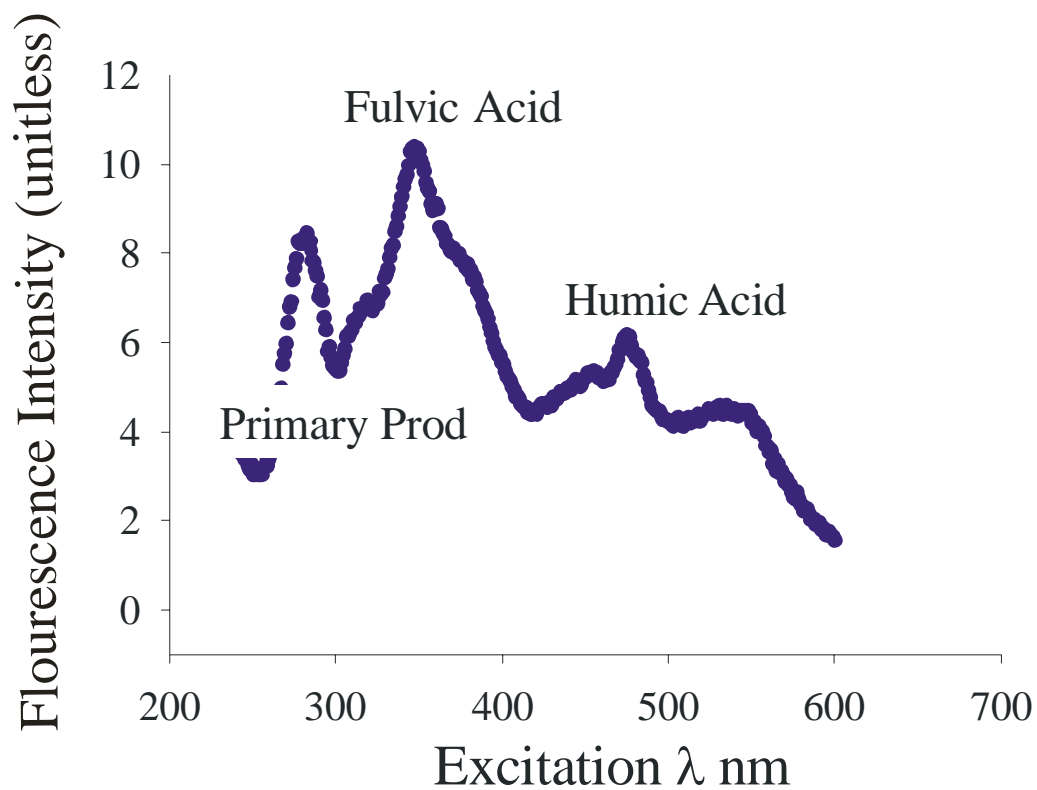


Figure 7. Synchronous fluorescence in an estuarine water sample. The excitation wave lengths are presented for primary production, fulvic acids and humic acids.

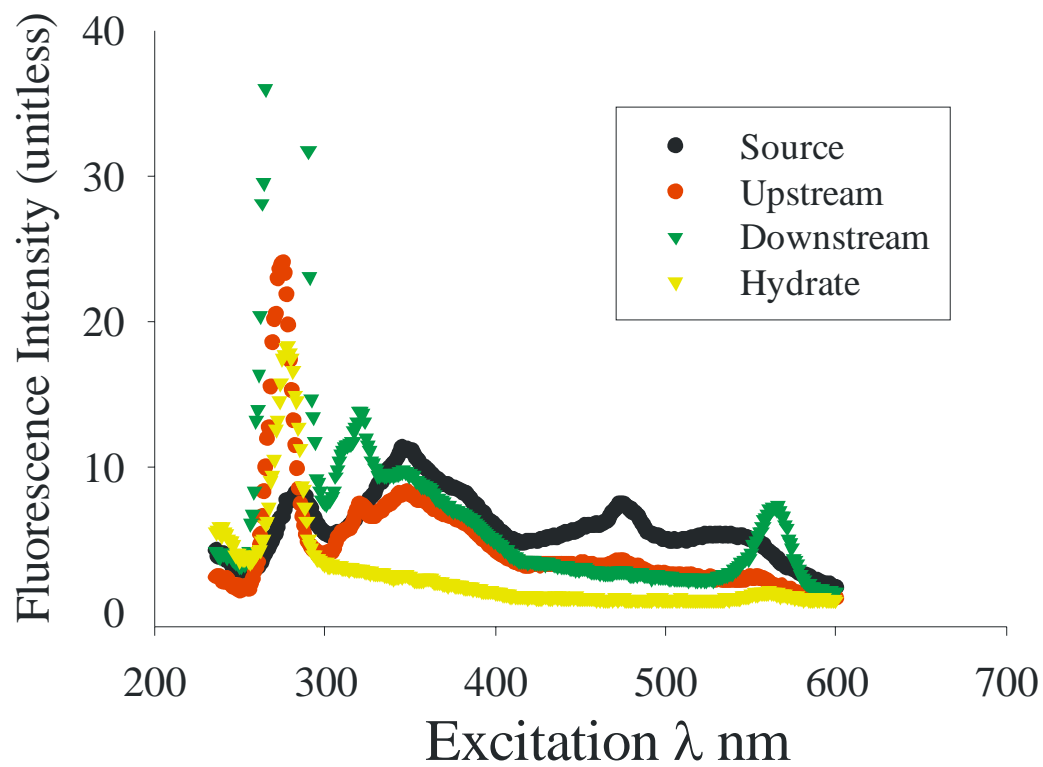


Figure 8. Emission spectra (fluorescence intensity) for organic matter in source, upstream, downstream and hydrate waters.

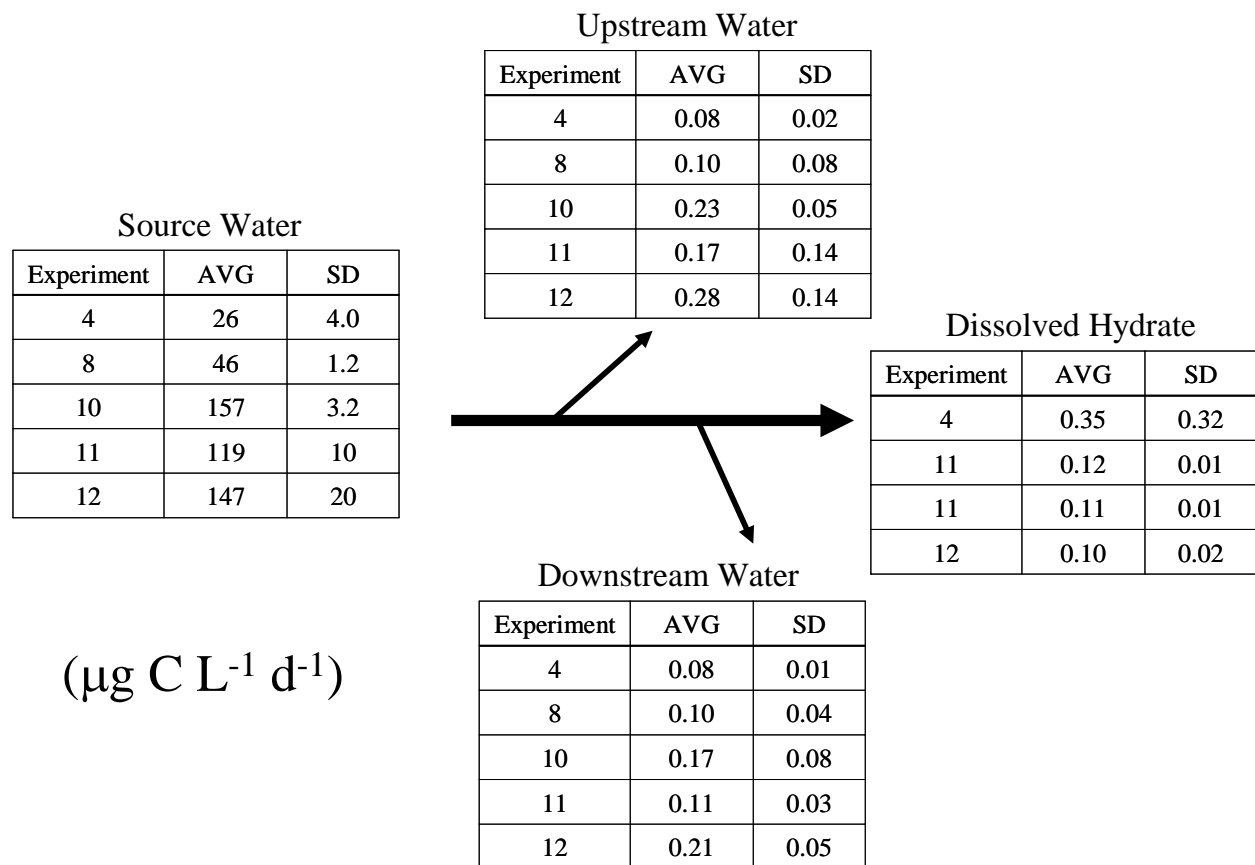


Figure 9. Heterotrophic bacterial production (average (AVG) and standard deviation (SD) $\mu\text{C L}^{-1} \text{ d}^{-1}$) compared in hydrate formation experiments between source, upstream, downstream and hydrate water.

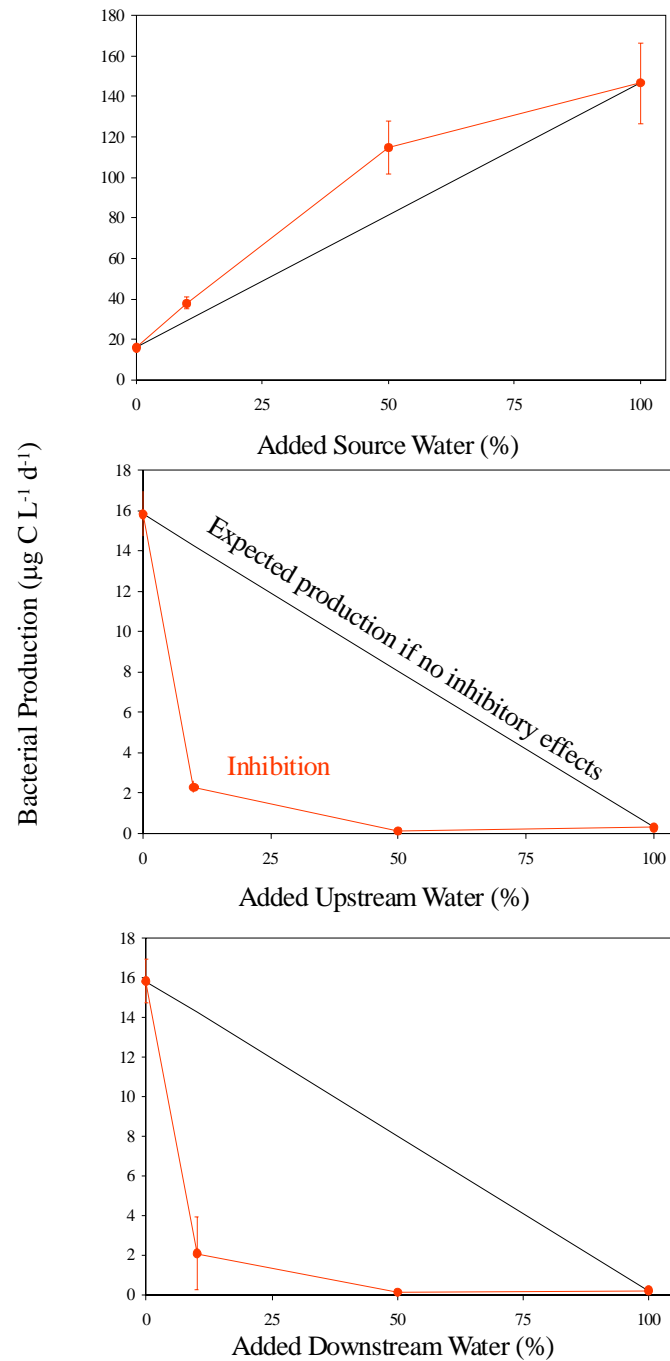


Figure 10. Heterotrophic bacterial production ($\mu\text{C L}^{-1} \text{d}^{-1}$) mixed with different percentages of Tampa Bay source water, upstream and downstream water.